Data Evaluation Report on the Acute Toxicity of KNF-S-474m (Metconazole) to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number {.....} EPA MRID Number 468084-28

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	329169
OECD Data Point	{.....}
EPA MRID	468084-28
EPA Guideline	OPPTS 850.4400 (123-2)

Test material: KNF-S-474m
Common name: Metconazole
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No.: 125116-23-6
Synonyms: None Provided

Purity: 97.9%
(83.8% *cis* isomer, 14.1% *trans* isomer)

Primary Reviewer: John Marton
Staff Scientist, Cambridge Environmental Inc.

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Date: 02/02/07

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Date: 6/13/07

6-13-07

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Date Evaluation Completed: 6/13/07

CITATION: Hoberg, James R. 2006. Metconazole (KNF-S-474m)- Toxicity to the Duckweed, *Lemna gibba*. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory report number 12709.6232. Study sponsored by Kureha Chemical Industry Company, Ltd., Tokyo, Japan. Study completed March 7, 2006.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants Duckweed (*Lemna gibba*) were exposed to KNF-S-474m (Metconazole) at nominal concentrations of 0 (negative and solvent controls), 0.00050, 0.0020, 0.0080, 0.032, 0.13 and 0.51 mg ai/L under static renewal conditions; mean-measured concentrations were <0.00025 (<LOQ; controls), 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L. The NOAEC and EC_{50} values based on frond number, the most sensitive endpoint, were 0.00051 and 0.022 mg ai/L, respectively. The % growth inhibition in the treated culture as compared to the control, based on fronds/rep, ranged from 8 to 87%. The % growth inhibition in the treated culture as compared to the control, based on growth rate, ranged from 2 to 63%. The % growth inhibition in the treated culture as compared to the control, based on biomass (dry weight), ranged from -4 to 72%.

By Day 7, fronds in the mean-measured 0.0085-0.57 mg ai/L treatment groups were observed to be curled. Curled fronds were first observed on Day 3 in the mean-measured 0.028-0.057 mg ai/L treatment groups and on Day 5 in the mean-measured 0.0085 mg ai/L treatment group. On Day 7, fronds in the mean-measured 0.13 and 0.57 mg ai/L treatment groups were noted as having less root formation than the control fronds.

This toxicity study is scientifically sound and is classified as ACCEPTABLE based on the guideline for an acute freshwater vascular plant toxicity study.

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static Renewal

Frond Number:
EC_{05}: 0.00048 mg ai/L * 95% C.I.: (0.00009, 0.0025)
EC_{50}: 0.022 mg ai/L 95% C.I.: 0.011-0.046 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.986±0.129

Growth Rate:
EC_{05}: 0.0010 mg ai/L 95% C.I.: 0.00015-0.0070 mg ai/L
EC_{50}: 0.13 mg ai/L 95% C.I.: 0.071-0.24 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.785±0.119

Biomass (Dry Weight):
EC_{05}: 0.00060 mg ai/L 95% C.I.: 0.000086-0.0041 mg ai/L
EC_{50}: 0.064 mg ai/L 95% C.I.: 0.032-0.13 mg ai/L
NOAEC: 0.0085 mg ai/L
Probit Slope: 0.809±0.117
Endpoint(s) Effected: Frond Number, Growth Rate and Biomass (Dry Weight)

*less than lowest concentration tested

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in U.S. EPA OPPTS Ecological Effects Test Guideline 850.4400, Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II, "Public Draft", EPA 712-C-96-156. The following deviations from OPPTS 850.4400 were noted:

1. The results of the periodic screening analysis of the dilution water were not reported; however, the study author reported that concentrations of particulate matter, metals, pesticides and chlorine were all within acceptable limits.
2. Analytical verification was only conducted before and after one renewal period. OPPTS guidance recommends that test solutions be analyzed before and after all renewals.

These deviations did not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice Regulations (40 CFR, Part 160) with the following exception: routine water contaminant screening analyses were conducted at GeoLabs, Inc., Braintree, MA using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92.

A. MATERIALS:

1. Test material KNF-S-474m (Metconazole)

Description: Not Reported

Lot No./Batch No. : AS2122a (Lot No.)

Purity: 97.9 % (83.8% *cis* isomer, 14.1% *trans* isomer)

Stability of compound under test conditions: Analytical verification of the test material was conducted on samples taken from new solutions on Day 3 and aged solutions on Day 5. Samples from the new solutions on Day 3 yielded recoveries of 88-300% of nominal. The 300% recovery was at the nominal 0.032 mg ai/L treatment group and was not believed to be representative of the actual exposure concentration and was therefore, excluded from the determination of mean-measured concentrations. Samples from the aged solutions on Day 5 yielded recoveries of 88-130% of nominal. The mean-measured concentrations represented values of 88-140% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored in a freezer.

Physicochemical properties of KNF-S-474m (Metconazole).

Parameter	Values	Comments
Water solubility at 20EC	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Name: Duckweed (*Lemna gibba*) *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: Not Provided
Source: In-house cultures (originally obtained from University of Toronto, Toronto, Canada)
Age of inoculum: Two days since previous transfer
Method of cultivation: 20X-AAP

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Two preliminary range-finding studies were conducted prior to definitive testing. The first range-finding test was conducted from February 4-11, 2005 with nominal concentrations of 0 (negative and solvent controls), 0.050, 0.50 and 5.0 mg ai/L, with two replicate vessels per control and treatment level. After 7 days of exposure, frond densities were 222 and 228 fronds/replicate in the negative and solvent controls, respectively, compared to 93, 51 and 40 fronds/replicate in the nominal 0.050, 0.50 and 5.0 mg ai/L treatment groups, respectively. Fronds in the treatment levels were observed to be slightly chlorotic and curled; fronds in the negative and solvent controls were observed to be slightly chlorotic.

The second range-finding test was conducted from February 24 to March 3, 2005 with nominal concentrations of 0 (negative and solvent controls), 0.0050, 0.050, 0.50 and 5.0 mg ai/L, with two replicate vessels per control and treatment level. After 7 days of exposure, frond densities were 338 and 288 fronds/replicate in the negative and solvent controls, respectively, compared to 264, 109, 54 and 37 fronds/replicate in the nominal 0.0050, 0.050, 0.50 and 5.0 mg ai/L treatment groups, respectively. Fronds in the controls and nominal 0.0050 mg ai/L treatment group were observed to be normal and fronds in the three remaining treatment groups were observed to be curled. Based on these results and consultation with the Study Monitor, a dilution factor of 33% was utilized and nominal concentrations of 0 (negative and solvent controls), 0.00080, 0.0024, 0.0072, 0.022, 0.065, 0.19 and 0.57 mg ai/L were used for definitive testing. However, after an unsuccessful first definitive test, a second definitive test was conducted with nominal concentrations of 0 (negative and solvent controls), 0.00050, 0.0020, 0.0080, 0.032, 0.13 and 0.51 mg ai/L. See Reviewer's Comments section for results from first definitive test.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Temperature and photoperiod were the same as test conditions; however, light intensity was 6500-8600 lux during the culture period and 4200-6700 lux during definitive testing. Not reported	
Test system Static/static renewal Renewal rate for static renewal	Static Renewal Test solutions were renewed on Days 3 and 5	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Temperature-controlled environmental chamber	
Duration of the test	7 Days	An additional 7 day exposure, followed by a 7 day recovery period, was conducted using a single nominal concentration of 0.010 mg ai/L and the solvent control. Three replicate vessels were established for both treatments and observations and frond counts were made on Days 3, 5 and 7. The 0.010 mg ai/L test solution was prepared from a 5.1 mg ai/mL stock solution. The 7 day recovery phase was initiated by transferring three to five plants totaling 15 fronds from each of the three replicates of the treatment and solvent control into fresh algal medium. The fronds were transferred to fresh medium on Days 3 and 5. *EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 270 mL 100 mL	
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP Medium 7.6-7.9 8.4-9.1 $FeCl_3 \cdot 6H_2O$ $Na_2EDTA \cdot 2H_2O$	The pH increased by ≥0.4 units at all treatment levels between renewal periods. *EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Well water, source of deionized water for algal growth medium Adjusted to 7.5±0.1 None reported 3.9 mg/L (Sept. 2006) 3.1 mg/L (Dec. 2006) Not Reported Not Reported Not Reported Not Reported	The results of the periodic screening analysis of the dilution water were not reported; however, the study author reported that concentrations of particulate matter, metals, pesticides and chlorine were all within acceptable limits. *EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*

Parameter	Details	Remarks *Criteria*
Indicate how the test material is added to the medium (added directly or used stock solution)	5.1 mg ai/mL stock solution	
Aeration or agitation	None	
Sediment used **(for rooted aquatic vascular plants)** Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
Number of replicates Control: Solvent control: Treatments:	 3 3 3	
Number of plants/replicate	5 plants	*EPA requires 5 plants.*
Number of fronds/plant	3 fronds per plant	*EPA requires 3 fronds per plant.*
Test concentrations Nominal: Measured:	 0 (negative and solvent controls), 0.00050, 0.0020, 0.0080, 0.032, 0.13 and 0.51 mg ai/L <0.00025 (<LOQ; controls), 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	Acetone (CAS No. 67-64-1); 0.10 mL/L	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	All exposure solution and QC samples were analyzed for *cis*- and *trans*-metconazole by automated injection using gas chromatography with nitrogen phosphorous detection (GC/NPD). Test solutions were analyzed at the beginning and at the end of one renewal period (Days 3 and 5).	A method validation study was conducted prior to test initiation and established average recoveries from seawater for *cis*- and *trans*-metconazole of 101±6.80% and 103±6.80%, respectively. The mean recovery for total metconazole concentration was 102±6.26%.
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24°C Continuous lighting 4000-5900 lux (PAR; 45-86 $\mu E/m^2/s$)	
Reference chemical (if used) name: concentrations:	 *cis*- and *trans*-metconazole analytical standards 0.000500, 0.0500 and 0.600 mg ai/L	The reference chemical was used to prepare calibration standards. A positive control was not used.
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Frond density (Day 7), growth rate (Days 0-7) and biomass (Days 0-7) (dry weight)	
Measurement technique for frond number and other end points	Visual count	
Observation intervals	Days 0, 3, 5 and 7	
Other observations, if any	See Inhibitory Effects	
Indicate whether there was an exponential growth in the control	Yes. Frond density was 392 fronds/replicate in the negative control at test termination.	
Were raw data included?	Replicate data were provided in summarized data tables.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By test termination, frond densities were 392 and 362 fronds/rep in the negative and solvent controls, respectively, and 345, 322, 307, 168, 51 and 50 fronds/rep in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. Compared to the pooled control, reductions in frond density were 8.4, 15, 18, 55, 87 and 87% in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. The study author's NOAEC, LOAEC and EC_{50} (and 95% C.I.) values were 0.00051, 0.0027 and 0.025 (0.022-0.028) mg ai/L, respectively.

By test termination growth rates were 0.46 and 0.45 day^{-1} in the negative and solvent controls, respectively, and 0.44, 0.43, 0.43, 0.34, 0.17 and 0.17 day^{-1} in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. Compared to the pooled control, reductions in growth rate were 3.3, 5.5, 5.5, 25, 63 and 63% in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. The study author's NOAEC, LOAEC and EC_{50} (and 95% C.I.) values were 0.00051, 0.0027 and 0.098 (0.091-0.11) mg ai/L, respectively.

By test termination frond dry weights were 48.93 and 50.90 mg in the negative and solvent controls, respectively, and 45.30, 47.53, 43.17, 27.50, 16.27 and 13.50 mg in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. Compared to the pooled control, reductions in frond dry weights were 9.3, 4.8, 14, 45, 67 and 73% in the mean-measured 0.00051, 0.0027, 0.0085, 0.028, 0.13 and 0.57 mg ai/L treatment groups, respectively. The study author's NOAEC, LOAEC and EC_{50} (and 95% C.I.) values were 0.0027, 0.0085 and 0.051 (0.031-0.070) mg ai/L, respectively.

All of the study author's toxicity values were determined using a pooled control.

By Day 7, fronds in the mean-measured 0.0085-0.57 mg ai/L treatment groups were observed to be curled. Curled fronds were first observed on Day 3 in the mean-measured 0.028-0.057 mg ai/L treatment groups and on Day 5 in the mean-measured 0.0085 mg ai/L treatment group. On Day 7, fronds in the mean-measured 0.13 and 0.57 mg ai/L treatment groups were noted as having less root formation the control fronds.

Table 3: Effect of KNF-S-474m (Metconazole) on frond number of Duckweed (*Lemna gibba*)

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial frond number/test solution (or other endpoint)	Frond Number at			
		3 Days	5 Days	7 Days	
				Frond Number	% Inhibition[a]
Negative control	15	69	164	392	N/A
Solvent control	15	73	170	362	8
0.00051 (0.0050)	15	54	138	345	12
0.0027 (0.0020)	15	65	174	322	18
0.0085 (0.0080)	15	54	119	307	22
0.028 (0.032)	15	45	83	168	57
0.13 (0.13)	15	35	52	51	87
0.57 (0.51)	15	33	41	50	87
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Reviewer-estimated percent inhibition compared to the negative control.
N/A- Not Applicable

Table 4: Effect of KNF-S-474m (Metconazole) on growth of Duckweed, *Lemna gibba*

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial frond number/test solution	Growth rate (days $^{-1}$, mean)	Growth rate % Inhibition[a]	Biomass, dry weight (mg, mean)	Biomass % Inhibition[a]
Negative control	15	0.46	N/A	48.93	N/A
Solvent control	15	0.45	2	50.90	-4
0.00051 (0.0050)	15	0.44	4	45.30	7
0.0027 (0.0020)	15	0.43	7	47.53	3
0.0085 (0.0080)	15	0.43	7	43.17	12
0.028 (0.032)	15	0.34	26	27.50	44
0.13 (0.13)	15	0.17	63	16.27	67
0.57 (0.51)	15	0.17	63	13.50	72
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] Reviewer-estimated percent inhibition compared to the negative control. Negative percent inhibition indicates promoted growth.
N/A- not applicable

Table 5: Statistical endpoint values.

Statistical Endpoint	Frond No.[a]	Growth Rate [a]	Biomass (Dry Weight) [a]
NOAEC (mg ai/L)	0.00051	0.00051	0.0027
LOAEC (mg ai/L)	0.0027	0.0027	0.0085
IC_{50} or EC_{50} (mg ai/L) (95% C.I.)	0.025 (0.022-0.028)	0.098 (0.091-0.11)	0.051 (0.031-0.070)
Other (IC_{05}/EC_{05})	<0.00051	0.0023 (0.0008-0.011)	<0.00051
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

[a] Toxicity values determined by the study author were based on analyses using the mean-measured concentrations and by comparing data to the pooled control

B. REPORTED STATISTICS:

The 7-Day EC_{05}, EC_{50} and EC_{90} values (and corresponding 95% confidence intervals) were determined using ToxStat® version 3.5. If no concentration resulted in a 5, 50 or 90% reduction, the EC values were empirically estimated to be greater than the highest concentration tested.

The data for the three endpoints were checked for normality using Shapiro-Wilks' Test and for homogeneity of variance using Bartlett's Test. As all data sets were normal and homogenous, the NOAEC and LOAEC values were determined using Williams' Test or Bonferroni's Test. A t-test was used to determine if significant differences existed between the negative and solvent controls for all three endpoints; since no differences were detected, the controls were pooled and all subsequent analyses were conducted using the pooled control. All toxicity values were determined using TOXSTAT version 3.5 and were based on the mean-measured concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): Prior to determining the NOAEC and LOAEC values for frond number, growth rate and frond biomass (dry weight), the reviewer analyzed the data from the negative and solvent controls using a Student's t-Test to determine if a significant difference existed between the two controls. No differences were detected, and all subsequent analyses were conducted using the negative control only. The data from each of the three endpoints were tested for normality using the Shapiro-Wilks and Chi-square tests and for homogeneity of variance using the Hartley and Bartlett's tests. Growth rate and dry weight met these assumptions of ANOVA; therefore, the NOAEC and LOAEC values were determined using the parametric Dunnett's and Williams' tests. The data for frond number did not meet these assumptions and the NOAEC and LOAEC values were determined using the non-parametric Kruskal-Wallis test. However, as the Kruskal-Wallis test did not detect any significant differences at any treatment levels, the reviewer visually determined the NOAEC value based on the percent reductions relative to the negative control. Reductions in the frond number were 8% at the measured 0.00051 mg ai/L treatment level and ≥12% at the remaining treatment levels. Therefore, the reviewer visually determined the NOAEC value for growth rate to be 0.00051 mg ai/L. The ECx values (and 95% C.I.) were determined using the probit analysis. NOAEC and LOAEC values were determined using Toxstat Statistical Software and the ECx values were determined using Nuthatch Statistical software. The mean-measured concentrations were used in all analyses.

Frond Number:
EC_{05}: 0.00048 mg ai/L * 95% C.I.: (0.00009, 0.0025)
EC_{50}: 0.022 mg ai/L 95% C.I.: 0.011-0.046 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.986±0.129

Growth Rate:
EC_{05}: 0.0010 mg ai/L 95% C.I.: 0.00015-0.0070 mg ai/L
EC_{50}: 0.13 mg ai/L 95% C.I.: 0.071-0.24 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.785±0.119

Biomass (Dry Weight):
EC_{05}: 0.00060 mg ai/L 95% C.I.: 0.000086-0.0041 mg ai/L
EC_{50}: 0.064 mg ai/L 95% C.I.: 0.032-0.13 mg ai/L
NOAEC: 0.0085 mg ai/L
Probit Slope: 0.809±0.117
*less than lowest concentration tested

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER=S COMMENTS:

The reviewer's results were determined by comparing treatment data to the negative control only; the study author used a pooled control. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

Only samples from one renewal period (Days 3 and 5) were analyzed for the test material, so it is impossible to determine time-weighted averages. Therefore, the mean-measured concentrations were used for determining the

toxicity values. The test material appeared to remain stable, so mean measured concentrations probably represented the actual exposure concentrations.

The stock solution was observed to be clear and colorless with no visible sign of undissolved test material present.

Analysis of the QC samples yielded recoveries of 82.4-113% of the nominal fortified levels (0.000500, 0.0500 and 0.600 mg ai/L).

An initial definitive test was conducted from March 18-28, 2005 at nominal concentrations of 0 (negative and solvent controls), 0.00080, 0.0024, 0.0072, 0.022, 0.065, 0.19 and 0.57 mg ai/L, with three replicate test vessels for each control and treatment level. After 7 days of exposure, frond density was 490 and 512 fronds/rep in the negative and solvent controls, respectively, compared to frond densities of 434, 428, 412, 273, 119, 93 and 72 fronds/rep in the nominal 0.00080, 0.0024, 0.0072, 0.022, 0.065, 0.19 and 0.57 mg ai/L treatment groups, respectively. Fronds in the 0.022, 0.065, 0.19 and 0.57 mg ai/L treatment group were observed to be curled, while fronds in the controls and remaining treatment levels appeared to be normal. Williams' Test determined the NOAEC value to be <0.00080 mg ai/L. Based on these results and consultation with the Study Sponsor, a second definitive test was conducted with nominal concentrations of 0 (negative and solvent controls), 0.00050, 0.0020, 0.0080, 0.032, 0.13 and 0.51 mg ai/L.

The additional 7 day exposure period was conducted with nominal concentrations of 0 (solvent control) and 0.010 mg ai/L under static renewal conditions. Three replicate test vessels were used for the solvent control and treatment level, and each vessel was renewed on Days 3 and 5. Analytical verification was only conducted on new solutions on Day 0 and aged solutions on Day 3. The resulting mean-measured concentrations were <0.00050 (<LOQ; solvent control) and 0.011 mg ai/L. The QC samples yielded recoveries of 92.9-117% of the nominal fortified levels (0.00500, 0.0100 and 0.020 mg ai/L). After 7 days of exposure frond density was 556 fronds/rep in the solvent control and 425 fronds/rep in the mean-measured 0.011 mg ai/L treatment level, indication a reduction of 24%; fronds in the treatment level were slightly curled. During the test the pH ranged from 7.6-8.2 in the new solutions and 8.3-9.3 in the aged solutions. Temperature was maintained at 24-25°C and light intensity ranged from 5100 to 5900 lux. After 7 days of exposure, the recovery phase was initiated by transferring three to five plants totaling 15 fronds from each of the three replicates of the treatment and solvent control into fresh untreated algal medium. The fronds were transferred to fresh medium on Days 3 and 5. After 7 days of recovery, frond density was 393 fronds/rep in the group from the previous solvent control and 413 frond/rep in the group from the previous mean-measured 0.011 mg ai/L treatment group, indication a 5% increase. These results demonstrated that the test organisms recovered from the initial exposure of 0.011 mg ai/L.

The experimental phase of the definitive toxicity test (including dry weight determinations) was conducted from September 16 to 26, 2005.

F. CONCLUSIONS:

The study is scientifically sound and is thus acceptable.

The 7-Day NOAEC, LOAEC and EC_{50} values for frond number, the most sensitive endpoint, were 0.00051, 0.0027 and 0.022 mg ai/L, respectively.

Frond Number:
EC_{05}: 0.00048 mg ai/L * 95% C.I.: (0.00009, 0.0025)
EC_{50}: 0.022 mg ai/L 95% C.I.: 0.011-0.046 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.986±0.129

Growth Rate:
EC_{05}: 0.0010 mg ai/L 95% C.I.: 0.00015-0.0070 mg ai/L
EC_{50}: 0.13 mg ai/L 95% C.I.: 0.071-0.24 mg ai/L
NOAEC: 0.00051 mg ai/L
Probit Slope: 0.785±0.119

Biomass (Dry Weight):
EC_{05}: 0.00060 mg ai/L 95% C.I.: 0.000086-0.0041 mg ai/L
EC_{50}: 0.064 mg ai/L 95% C.I.: 0.032-0.13 mg ai/L
NOAEC: 0.0085 mg ai/L
Probit Slope: 0.809±0.117
Endpoint(s) Effected: Frond Number, Growth Rate and Biomass (Dry Weight)

*less than lowest concentration tested

III. REFERENCES:

ASTM. 2002. Standard Guide for Conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19426.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Fronds/replicate; Day 7, mg ai/L
File: 8428fd Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	391.6667	CALCULATED t VALUE =	0.6303
GRP2 (BLANK CRTL) MEAN =	362.3333	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	29.3333		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

Fronds/replicate; Day 7, mg ai/L
File: 8428fd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	7	7	0

Calculated Chi-Square goodness of fit test statistic = 4.3919
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Fronds/replicate; Day 7, mg ai/L
File: 8428fd Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 15249.333

W = 0.862

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Fronds/replicate; Day 7, mg ai/L
File: 8428fd Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 157.13
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

```
Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


Fronds/replicate; Day 7, mg ai/L
File: 8428fd       Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
------------------------------------------------------------------------------

Calculated B statistic =   16.94
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
------------------------------------------------------------------------------

Data FAIL homogeneity test at 0.01 level. Try another transformation.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

Fronds/replicate; Day 7, mg ai/L
File: 8428fd       Transform: NO TRANSFORMATION

              KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
------------------------------------------------------------------------------
                                TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION           MEAN          ORIGINAL UNITS         SUM
-----  --------------------    -----------   ------------------  -----------
  1             neg control      391.667           391.667          54.000
  2                 0.00051      345.333           345.333          50.000
  3                  0.0027      322.000           322.000          45.000
  4                  0.0085      307.333           307.333          37.000
  5                   0.028      168.000           168.000          24.000
  6                    0.13       50.667            50.667          10.500
  7                    0.57       50.333            50.333          10.500
------------------------------------------------------------------------------

  Calculated H Value =  17.184         Critical H Value Table =  12.590
  Since  Calc H > Crit H  REJECT Ho:All groups are equal.


Fronds/replicate; Day 7, mg ai/L
File: 8428fd       Transform: NO TRANSFORMATION

 DUNNS MULTIPLE COMPARISON -  KRUSKAL-WALLIS  -  TABLE 2 OF 2
------------------------------------------------------------------------------
                                                    GROUP
                      TRANSFORMED  ORIGINAL   0 0 0 0 0 0 0
```

GROUP	IDENTIFICATION	MEAN	MEAN	7 6 5 4 3 2 1
7	0.57	50.333	50.333	\
6	0.13	50.667	50.667	. \
5	0.028	168.000	168.000	. . \
4	0.0085	307.333	307.333	. . . \
3	0.0027	322.000	322.000	. . . . \
2	0.00051	345.333	345.333	. . . . . \
1	neg control	391.667	391.667	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 5.065

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.00048	9.2E-05	0.0025	0.34	0.19
EC10	0.0011	0.00027	0.0047	0.29	0.24
EC25	0.0046	0.0016	0.013	0.22	0.34
EC50	0.022	0.011	0.046	0.15	0.49

Slope = 0.986 Std.Err. = 0.129

!!!Poor fit: p = 0.0011 based on DF= 4.0 14.

8428FD : Fronds/replicate; Day 7, mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	392.	390.	1.63	100.	0.00
0.000510	3.00	345.	369.	-24.1	94.7	5.29
0.00270	3.00	322.	319.	3.38	81.7	18.3
0.00850	3.00	307.	257.	49.9	66.0	34.0
0.0280	3.00	168.	180.	-11.8	46.1	53.9
0.130	3.00	50.7	87.8	-37.1	22.5	77.5
0.570	3.00	50.3	32.2	18.1	8.26	91.7

!!!Warning: EC5 not bracketed by doses evaluated.

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN	= 0.4600	CALCULATED t VALUE =	0.5477
GRP2 (BLANK CRTL) MEAN	= 0.4500	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS	= 0.0100		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	9	5	7	0

Calculated Chi-Square goodness of fit test statistic = 7.6969
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.005

W = 0.969

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

Data PASS normality test at P=0.01 level. Continue analysis.

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 27.00
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 5.64

```
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
---------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr        Transform: NO TRANSFORMATION

                              ANOVA TABLE
---------------------------------------------------------------------------

SOURCE             DF              SS                MS              F
---------------------------------------------------------------------------
Between             6              0.2902          0.0484        121.000

Within (Error)     14              0.0051          0.0004
---------------------------------------------------------------------------
Total              20              0.2953
---------------------------------------------------------------------------

  Critical F value =   2.85  (0.05,6,14)
  Since  F > Critical F  REJECT  Ho:All groups equal


Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 1 OF 2            Ho:Control<Treatment
---------------------------------------------------------------------------

                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION         MEAN          ORIGINAL UNITS    T STAT  SIG
-----  --------------------   ----------   ------------------  ------  ---
  1            neg control      0.460            0.460
  2                0.00051      0.443            0.443          1.021
  3                 0.0027      0.430            0.430          1.837
  4                 0.0085      0.427            0.427          2.041
  5                  0.028      0.343            0.343          7.144  *
  6                   0.13      0.173            0.173         17.555  *
  7                   0.57      0.170            0.170         17.759  *
---------------------------------------------------------------------------
Dunnett table value =  2.53     (1 Tailed Value, P=0.05,  df=14,6)


Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr        Transform: NO TRANSFORMATION

      DUNNETTS TEST   -   TABLE 2 OF 2            Ho:Control<Treatment
---------------------------------------------------------------------------

                          NUM OF   Minimum Sig Diff  % of      DIFFERENCE
GROUP    IDENTIFICATION   REPS     (IN ORIG. UNITS)  CONTROL   FROM CONTROL
-----  --------------------  -------  ----------------  -------  ------------
  1            neg control     3
  2                0.00051     3            0.041        9.0         0.017
```

3	0.0027	3	0.041	9.0	0.030
4	0.0085	3	0.041	9.0	0.033
5	0.028	3	0.041	9.0	0.117
6	0.13	3	0.041	9.0	0.287
7	0.57	3	0.041	9.0	0.290

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	0.460	0.460	0.460
2	0.00051	3	0.443	0.443	0.443
3	0.0027	3	0.430	0.430	0.430
4	0.0085	3	0.427	0.427	0.427
5	0.028	3	0.343	0.343	0.343
6	0.13	3	0.173	0.173	0.173
7	0.57	3	0.170	0.170	0.170

Growth rate (days^-1); Days 0-7, mg ai/L
File: 8428gr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.460				
0.00051	0.443	1.076		1.76	k= 1, v=14
0.0027	0.430	1.936	*	1.85	k= 2, v=14
0.0085	0.427	2.152	*	1.88	k= 3, v=14
0.028	0.343	7.531	*	1.89	k= 4, v=14
0.13	0.173	18.504	*	1.90	k= 5, v=14
0.57	0.170	18.719	*	1.91	k= 6, v=14

s = 0.019
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.0010	0.00015	0.0070	0.39	0.15
EC10	0.0030	0.00062	0.015	0.33	0.21
EC25	0.018	0.0062	0.052	0.22	0.34
EC50	0.13	0.071	0.24	0.13	0.54

Slope = 0.785 Std.Err. = 0.119

!!!Poor fit: p < 0.001 based on DF= 4.00 14.0

8428GR : Growth rate (days^-1); Days 0-7, mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	0.460	0.470	-0.00986	100.	0.00
0.000510	3.00	0.443	0.456	-0.0127	97.1	2.95
0.00270	3.00	0.430	0.426	0.00401	90.7	9.34
0.00850	3.00	0.427	0.387	0.0397	82.4	17.6
0.0280	3.00	0.343	0.329	0.0147	70.0	30.0
0.130	3.00	0.173	0.235	-0.0615	50.0	50.0
0.570	3.00	0.170	0.144	0.0257	30.7	69.3

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	48.9333	CALCULATED t VALUE =	-0.4102
GRP2 (BLANK CRTL) MEAN =	50.9000	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	-1.9667		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	8	0

Calculated Chi-Square goodness of fit test statistic = 5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 229.987

W = 0.963

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873

Data PASS normality test at P=0.01 level. Continue analysis.

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 784.62
Closest, conservative, Table H statistic = 1705.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 2
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
but do not differ greatly, the Hartley test may still be used
as an approximate test (average df are used).

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 15.40
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	4176.973	696.162	42.377
Within (Error)	14	229.987	16.428	
Total	20	4406.960		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	48.933	48.933		
2	0.00051	45.300	45.300	1.098	
3	0.0027	47.533	47.533	0.423	
4	0.0085	43.167	43.167	1.743	
5	0.028	27.500	27.500	6.477	*
6	0.13	16.267	16.267	9.871	*
7	0.57	13.500	13.500	10.707	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	3			
2	0.00051	3	8.373	17.1	3.633
3	0.0027	3	8.373	17.1	1.400
4	0.0085	3	8.373	17.1	5.767
5	0.028	3	8.373	17.1	21.433
6	0.13	3	8.373	17.1	32.667
7	0.57	3	8.373	17.1	35.433

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	3	48.933	48.933	48.933
2	0.00051	3	45.300	45.300	46.417
3	0.0027	3	47.533	47.533	46.417
4	0.0085	3	43.167	43.167	43.167
5	0.028	3	27.500	27.500	27.500
6	0.13	3	16.267	16.267	16.267
7	0.57	3	13.500	13.500	13.500

Frond dry weight (mg); Days 0-7, mg ai/L
File: 8428dw Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	48.933				
0.00051	46.417	0.760		1.76	k= 1, v=14
0.0027	46.417	0.760		1.85	k= 2, v=14
0.0085	43.167	1.743		1.88	k= 3, v=14
0.028	27.500	6.477	*	1.89	k= 4, v=14
0.13	16.267	9.871	*	1.90	k= 5, v=14
0.57	13.500	10.707	*	1.91	k= 6, v=14

s = 4.053
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.00060	8.6E-05	0.0041	0.40	0.14
EC10	0.0017	0.00033	0.0086	0.34	0.20
EC25	0.0094	0.0030	0.030	0.24	0.32
EC50	0.064	0.032	0.13	0.14	0.50

Slope = 0.809 Std.Err. = 0.117

!!!Poor fit: p = 0.0070 based on DF= 4.0 14.

8428DW : Frond dry weight (mg); Days 0-7, mg ai/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	48.9	50.2	-1.27	100.	0.00
0.000510	3.00	45.3	48.0	-2.67	95.5	4.46
0.00270	3.00	47.5	43.5	3.98	86.7	13.3
0.00850	3.00	43.2	38.2	4.93	76.2	23.8
0.0280	3.00	27.5	30.9	-3.38	61.5	38.5
0.130	3.00	16.3	20.2	-3.95	40.3	59.7
0.570	3.00	13.5	11.1	2.36	22.2	77.8